



A Comparison of Leaf Anatomy in Field-grown *Gossypium hirsutum* and *G. barbadense*

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Gossypium hirsutum L. (upland cotton) and *G. barbadense* L. (Pima cotton) are two of the most important fibre producing cotton species in cultivation. When grown side-by-side in the field, *G. hirsutum* has higher photosynthetic and transpiration rates (Lu *et al.*, 1997, *Australian Journal of Plant Physiology* 24: 693–700). The present study was undertaken to determine if the differences in physiology can be explained by leaf and canopy morphology and anatomy. Scanning electron microscopy was used to compare the leaf anatomy of field-grown upland ('Delta' and 'Pine Land 50') and Pima ('S6') cotton. Compared to *G. hirsutum*, mature leaves of *G. barbadense* are larger and thinner, with a thinner palisade layer. *G. barbadense* leaves show significant cupping or curling which allows for a more even absorption of insolation over the course of the day and much more light penetration into the canopy. Although *G. barbadense* leaves have a 70–78 % higher stomatal density on both the abaxial and the adaxial surfaces, its stomates are only one third the size of those of *G. hirsutum*. This results in *G. barbadense* having only about 60 % of the stomatal surface area per leaf surface area compared to *G. hirsutum*. These results are indicative of the anatomical and physiological differences that may limit the yield potential of *G. barbadense* in certain growing environments.

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INTRODUCTION

Cotton (*Gossypium* spp.) is a perennial dicot in the family Malvaceae. Through intensive breeding programmes, cultivars have been developed that are grown commercially as annuals. *G. hirsutum*, the major species grown in the USA, has been extensively bred over the years, resulting in greatly enhanced performance. Overall, yield improvements have resulted in more reproductive structures and fewer vegetative structures (Meredith and Bridge, 1973). Another commercial cotton, *G. barbadense* ('Pima'), has superior fibre properties that make it particularly promising for cotton production. The increased price paid for the lint reflects these enhanced fibre properties. However, yields for *G. barbadense* are less than those of *G. hirsutum*. The lower *G. barbadense* yields, together with a greater sensitivity to suboptimal growth conditions and the need for a longer growing season restrict the production of this higher quality cotton. Breeding programmes directed at incorporating the desirable fibre traits of *G. barbadense* into *G. hirsutum* have failed thus far to produce commercially viable genotypes.

Although *G. barbadense* and *G. hirsutum* are closely related species, their centres of origin differ geographically and ecologically (Hutchinson *et al.*, 1947; Percy and Wendel, 1990; Wendel *et al.*, 1992). It is therefore not unreasonable to assume that cultivars of the two species

would retain distinctive anatomical, morphological, and physiological traits related to their environmental origins. Examples of divergent traits between the species do exist. At the level of gross morphology, the species differ in such traits as locule number, bract teeth number and size, number of seeds per locule, degree of seed fuzz, and single gene traits such as flower and pollen colour (Fryxell, 1984). Whereas *G. hirsutum*, as a species, is strongly heliotrophic, this trait is absent or weakly expressed in *G. barbadense* (pers. obs.). Likewise, the day neutral flowering response appears to be a simply inherited trait in *G. barbadense* (Lewis and Richmond, 1960), but a complexly inherited trait in *G. hirsutum* (Kohel and Richmond, 1962). Susceptibility or resistance to various diseases have also been reported to vary between the two species (Bell, 1984).

Complicating the assignment of traits to one species or the other in improved cultivars is the fact that introgressive breeding has been practised between the species; primarily through incorporation of portions of the *G. hirsutum* genome into *G. barbadense*. The degree to which *G. hirsutum* traits have been incorporated into *G. barbadense* can be over-emphasized, however. The last documented introgressive event contributing to the American Pima germplasm pool occurred over 45 years ago (Feaster and Turcotte, 1962). Since that time there have been generations of selection within *G. barbadense* to recover the characteristics of that species' phenotype. This has been coupled with a reported natural tendency

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for selective elimination of donor parent genotypes in interspecific backcrosses (Stephens, 1949). In a molecular analysis of *G. hirsutum* introgression into Pima (*G. barbadense*) cotton, 7.3 % of the alleles of Pima cultivars were found to be derived from *G. hirsutum* (Wang *et al.*, 1995). These alleles were not randomly distributed within the *G. barbadense* genome, since nearly 60 % of the total introgression was found within five specific chromosomal regions accounting for less than 10 % of the genome. The non-random conservation of specific *G. hirsutum* chromosomal regions in *G. barbadense* argues for the retention of very specific *G. hirsutum* traits within *G. barbadense*, and against the assumption that traits varying between representative cultivars of the two species are of a single species' origin. Despite numerous introgressive breeding events having been reported in *G. hirsutum* (Meredith, 1991), there is little evidence of the retention of significant portions of the *G. barbadense* genome in upland cultivars (Brubaker *et al.*, 1993). One allozyme investigation analysing 50 loci in 50 upland cultivars found a total lack of unique alleles among the cultivars and found only one putative *G. barbadense* allele in two cultivars (Wendel *et al.*, 1992).

Acting upon the inherent genetic and phenotypic differences between the two species has been nearly 100 years of breeding and selection for adaptation to two very different production environments. Whereas *G. hirsutum* has been bred primarily to maximize its performance in the rain-fed southeast of America, Pima cottons have been bred for performance under irrigation in the southwest. These selection patterns may have enhanced some trait differences between the species, while eliminating others. Despite known differences between the species, literature on anatomical and physiological differences is sparse. Unfortunately, in the past, there has been a tendency to apply agronomic and cultural knowledge of one species (*G. hirsutum*) to the other (*G. barbadense*) without verification of its applicability.

Crop production research efforts have been directed towards optimizing production through enhanced canopy carbon uptake. The availability of substrates (CO_2 and sunlight) together with the health of the photosynthetic machinery determine the carbon fixation potential. Within a complex system such as an agronomic crop canopy, multiple processes contribute to potential limitations in the optimal uptake and utilization of sunlight. Therefore, as the primary photosynthetic organs for plants, leaves play an essential role in the growth, development, and yield of a crop. The importance of that role for individual leaves varies over the time course of leaf and canopy development. In general, after an initial rapid rise in CO_2 fixation rate, photosynthesis declines with leaf age on both a whole canopy (Wells *et al.*, 1986; Peng and Kreig, 1991) and an individual leaf basis (Davis and McCree, 1978; Constable and Rawson, 1980; Kennedy and Johnson, 1981; Wulfschleger and Oosterhuis, 1990). The decline is related to a decrease in photosynthetic biochemical efficiency and to a decrease in irradiance caused by expanding upper canopy leaves (Sassenrath-Cole, 1995; Sassenrath-Cole *et al.*, 1996).

A typical pattern of leaf development and anatomical structure has been described for mesophytic C_3 plants

(Esau, 1965). The internal structure has a densely packed palisade mesophyll layer of cells on the adaxial side, and a more open layer of spongy mesophyll cells on the abaxis. This structural differentiation relates to the differences in physiological function between the two cell types within the leaf (Vogelmann *et al.*, 1996). Palisade parenchyma cells have numerous chloroplasts and maximize the interception and utilization of available sunlight. The more open cells of the lower leaf layer are better suited to favour the diffusion of CO_2 . The rationale has long been held that by partially separating the physiological functions through differences in anatomy, mesophytic C_3 leaves optimize the interception and utilization of available sunlight, maximize CO_2 diffusion into the leaf tissue, and minimize transpirational water loss.

This study was undertaken to compare gross leaf anatomy between *G. hirsutum* and *G. barbadense*. Anatomical differences may influence the observed differences in leaf physiology, microclimate, and canopy function. Leaf structure was examined to determine if changes in leaf thickness, stomatal differentiation, and stomatal distribution contribute to the reported changes in photosynthesis during leaf and canopy maturation in field-grown cotton.

MATERIALS AND METHODS

Growth conditions

Cotton seeds (*Gossypium hirsutum*, L. 'Delta' and 'Pine Land 50' and *G. barbadense* 'Pima S6') were planted in a well drained sandy loam in 1 m rows in 12 by 12 m plots at Mississippi State, USA on 3 May 1995. Plants were thinned by hand at the first true leaf stage to approx. ten plants per m row. Nitrogen was applied at 5.6 g m^{-2} at planting, followed by 4.5 g m^{-2} at mid-season. Potassium was broadcast 35 d prior to planting at a rate of 6.7 g m^{-2} . Standard agricultural practices were followed for weed and insect control.

Leaf tagging and harvest

Leaves for physiological and anatomical study were tagged at the quarter size with small jeweller's tags on 6 July. Initial harvest began that day, proceeded at 3–4 d intervals for 5 weeks, and continued at weekly intervals until leaf senescence on 28 October. Entire leaves were harvested in the morning, and transported to the lab in plastic bags over ice. An estimate of the degree of leaf cupping was made by measuring both the leaf area and leaf shadow using an image analyser system (Ikegami area meter, Ikegami Tsushinki Co., Ltd., Utsunomiya, Japan) as described previously (Sassenrath-Cole, 1995). The 'shadow' of the leaf in its natural configuration was measured as the projected two-dimensional area when the leaf was illuminated from above with a light source perpendicular to the plane of the leaf midrib. After measurement of the leaf shadow, the leaf was flattened and the total leaf area was determined. For anatomical studies we sampled two cross-sections of each leaf, a 'horizontal' and a 'vertical' sample, taken to

represent the different orientations of portions of the cupped leaf. The mid-section of each leaf lobe was defined as the 'horizontal' sample, and the section of leaf near the edge of the lobe was defined as the 'vertical' sample. These labels reflect the actual leaf orientation for *G. barbadense*, though the *G. hirsutum* leaves were not nearly as cupped.

Scanning electron microscopy

The central section of each leaf was fixed in fresh FAA (50 ml 95 % (v:v) ethanol, 5 ml glacial acetic acid, 10 ml 37 % (v:v) formaldehyde, 35 ml H₂O) for 24 h and rinsed in water for 3 d. Small (2 × 20 mm) strips were excised from appropriate regions of the leaves, cut into 2 mm squares and dehydrated in a graded ethanol series to 70 % alcohol. Tissues were frozen in liquid nitrogen and cracked with a pre-cooled razor blade. Ethanolic dehydration was then completed and samples were critically point dried with CO₂, sputter coated with Au, and viewed in an Hitachi 2460N SEM. Microscope magnification was calibrated using a magnification test specimen (Ladd Res. Ind.) and images were taken under standard conditions (25 kV, 20 mm working distance, and a standard spot size).

Morphometric measurement—leaf thickness

Leaf palisade mesophyll thickness, spongy mesophyll thickness and total leaf thickness were measured from SEMs of leaf cross-sections.

Morphometric measurement—stomatal densities

To quantify stomatal densities with the SEM, five areas of the abaxial and adaxial surfaces of both cotton species for each of the 11 collection periods were photographed using a video printer. Each area photographed represented 0.291 µm² (the field of view at a magnification of 200×). The number of stomata were counted directly from the prints and expressed on a per µm² of leaf surface area basis.

Morphometric measurement—stomatal dimensions

Stomatal dimensions were measured from similar video prints taken at 2000×. Ten stomata were measured from the adaxial and abaxial surfaces from each of three different mature leaves for each species. Because no difference was found in stomatal dimensions between the abaxial and adaxial surfaces, those data were pooled (therefore, $n = 60$ for each species, see Table 1). Stomatal length was defined as the length of the long axis of the area bounded by the outer stomatal ledges; width was the length of the short axis between the edges of the outer ledges. Stomatal area (A) was calculated for each stoma from length and width measurements, assuming that the opening between the outer stomatal ledges was a perfect ellipse and using the equation $A = \pi ab$, where a and b are 1/2 length and 1/2 width, respectively. We recognize that this area bounded by the outer stomatal ledges represented the maximum possible stomatal opening, and may bear no relationship to the actual stomatal opening at the time of tissue fixation.

Photon flux density

The photon flux density incident to the surface of the leaves was determined using small (1.7 mm²) gallium arsenide phosphide photodiodes placed directly on the adaxial leaf surfaces (Gutschick *et al.*, 1985). These GaAsP sensors have a sensitivity range in the photosynthetically active region. In addition, their small size and light weight make them ideal for recording PPFD incident to the leaf surface. Five to seven leaves from each cultivar were chosen at several times during the growing season for measurements. Three GaAsP sensors were placed on each leaf, near the midrib, and on each edge of the large central leaf lobe. Sensors were placed on the adaxis of each of the leaf areas, vertical and horizontal, to record the differences in insolation levels as a result of the orientation of the leaf surface. The data shown in Fig. 5 were collected on 23 Jul. 1995.

Photosynthesis

Individual leaf photosynthetic rates were measured on young, fully expanded leaves on intact plants in the field under ambient CO₂ using a LiCor 6200 gas exchange system with a 1 l cuvette (Li-Cor Inc, Lincoln, NE, USA). One leaf from each of five plants for each species was measured on 23 Jul. 1995. Equal aged, mature leaves were used. The response of photosynthesis to incident photon flux density was determined by exposing the leaves to different PPFD levels by placing shade cloths over the leaves (Sassenrath-Cole *et al.*, 1996). Leaves were allowed to equilibrate at each PPFD level for a minimum of 30 min prior to the determination of photosynthesis.

RESULTS

Cotton leaves expanded rapidly in both *G. hirsutum* and *G. barbadense*, reaching a maximum area approx. 20 d after the initiation of unfolding (Fig. 1A). *G. barbadense* leaves were 39 % larger at maturity than those of *G. hirsutum* (Fig. 1A), consistent with the leaf area measurements of Lu *et al.* (1997) who found a 45 % difference. Leaf thickness also increased during leaf development and continued to increase even after the cessation of area expansion. At maturity, *G. hirsutum* leaves were 50 % thicker than those of *G. barbadense* (Fig. 1B). The palisade layer in *G. hirsutum* accounted for much of the increase in thickness (Fig. 1C and D). These developmental differences were visually apparent in the examination of leaf cross-sections (Fig. 2). Differences in leaf thickness between days 7 and 57 were due to cell expansion. The difference between the two cottons in terms of leaf anatomy is even more apparent when the leaf area and thickness data are plotted against one another. *G. hirsutum* can be seen to have smaller, thicker leaves than *G. barbadense* (Fig. 3).

Leaves from the two species varied greatly in the degree of three-dimensional curvature (Fig. 4). *G. hirsutum* leaves remained nearly flat throughout development (i.e. the ratio of leaf area to leaf shadow approximated unity), while *G. barbadense* leaves showed significant cupping by day 10.

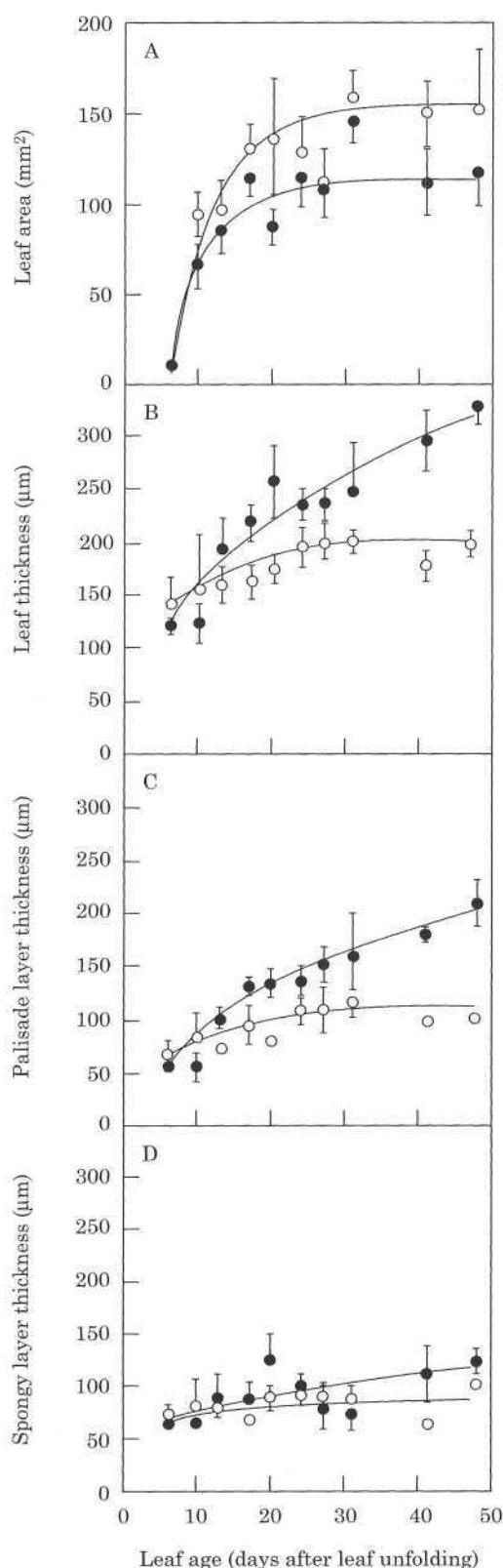


FIG. 1. Anatomical changes during leaf development in *G. hirsutum* (●) and *G. barbadense* (○) as a function of leaf age (i.e. days after unfolding). A, Leaf area; B, leaf thickness; C, palisade layer thickness; D, spongy mesophyll layer thickness. Mean \pm s.d., $n = 5$ for each datum point.

This three-dimensional cupping in *G. barbadense* was evidenced by a leaf shadow that was only half that of the actual leaf area by 15–20 d after unfolding (Fig. 4). Cupping relaxed slightly after completion of leaf expansion (approx. day 20), but remained significantly greater for *G. barbadense* than for *G. hirsutum* leaves.

Diurnal incident photon flux density was measured by placing small light sensors directly on the adaxial leaf surface. For *G. hirsutum*, which has a flat leaf, such measurements merely reflected changes in the daily solar angle (Fig. 5A). Due to leaf cupping of *G. barbadense* however, the direction the cupped leaf margin faced had a large impact on insolation. The east-facing adaxis received direct sunlight in the morning, while a west-facing adaxis received direct sunlight in the afternoon (Fig. 5B). Although not measured, it is also apparent that the opposite would hold for the abaxis: i.e. an east-facing abaxis would be illuminated in the afternoon and a west-facing abaxis in the morning.

From measured values of leaf surface orientation relative to horizontal, potential insolation to both leaf surfaces can be predicted over the course of the day (Herbert, 1983). A model leaf whose midrib is parallel to the ground and oriented along a north/south axis, would have five zones of interest: $0^{\circ}_{\text{adaxial}}$, $+90^{\circ}_{\text{adaxial}}$, $-90^{\circ}_{\text{adaxial}}$, $+90^{\circ}_{\text{abaxial}}$, and $-90^{\circ}_{\text{abaxial}}$ (see inset, Fig. 6). The vertical leaf margins ($+90^{\circ}$ and -90°) would receive direct sunlight only at sunrise and sunset (Fig. 6). As the solar zenith angle increases, PPFD to the leaf surface increases (Fig. 6). Portions of the leaf at different orientations to horizontal receive different levels of sunlight as a function of the relative solar angle over the course of the day.

Stomates on leaves of both species were normal in appearance (images not shown) and stomatal densities were higher on abaxial than adaxial surfaces (Fig. 7A, B, Table 1), as is common to C_3 dicots (Esau, 1965). *G. barbadense* had more stomates than *G. hirsutum* at all leaf ages (Fig. 7C), but their average maximum stomatal size was only 36 % of that of *G. hirsutum* (Table 1). The product of stomatal density and stomatal area ($\times 100$) yields the percent of leaf surface occupied by stomata. This calculation shows that *G. hirsutum* had 60 % more stomatal area per leaf area on both leaf surfaces (Table 1). Stomatal densities decreased on both leaf surfaces as the leaves expanded (Fig. 7C), most probably due to a 'dilution' effect as leaf area increased and no new stomates were formed. In spite of the large differences in leaf anatomy, photosynthesis in the two cotton species was identical under light-limiting conditions and only showed minor differences at light saturation (Fig. 8).

DISCUSSION

G. barbadense and *G. hirsutum* are the two *Gossypium* species utilized for commercial production of cotton fibre. *G. barbadense* fibres have more desirable characteristics for the textile industry, being notably longer and finer than those of *G. hirsutum*. However, *G. hirsutum* produces a greater yield than *G. barbadense*. While *G. barbadense* performs well in areas of the US Cotton Belt that

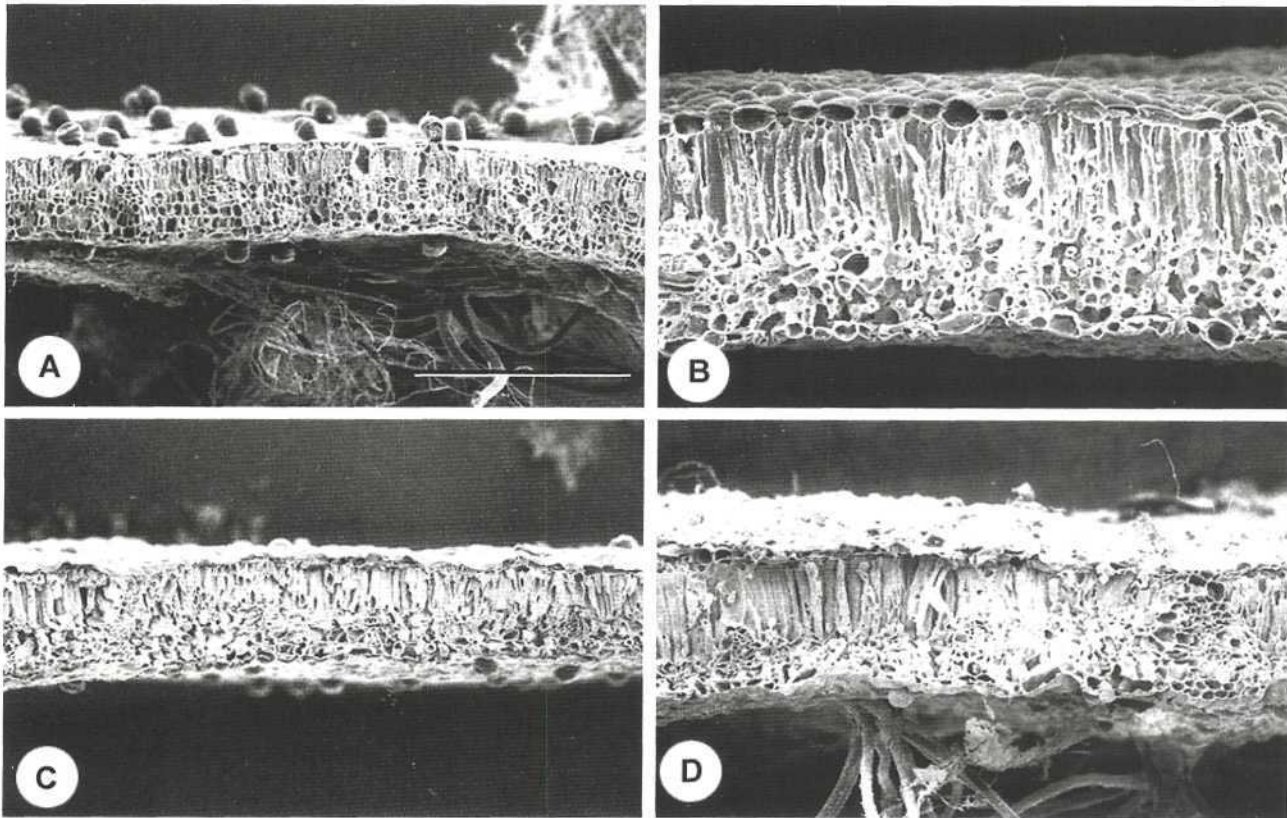


FIG. 2. Scanning electron micrographs of cross sections of *G. hirsutum* (A, B) and *G. barbadense* (C, D) leaves at 7 DAU (A, C) and 57 DAU (B, D). Scale bar in A = 250 μm for all panels.

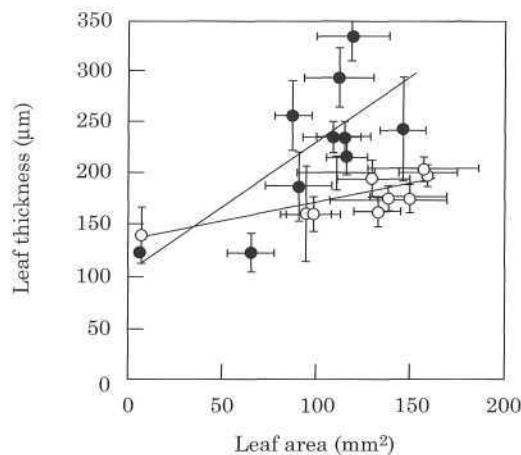


FIG. 3. Leaf thickness as a function of leaf area for *G. hirsutum* (●) and *G. barbadense* (○). Changes in leaf thickness measured from scanning electron micrographs are plotted as a function of the increasing leaf area upon leaf expansion. Mean \pm s.d., $n = 5$ for each datum point in both directions. Lines represent a least squares fit to the data sets.

experience high insolation levels and a long growing season, such as found in Arizona, the yield potential is significantly limited in other areas of the country. Both physiological and anatomical traits may contribute to the limited productivity of *G. barbadense*.

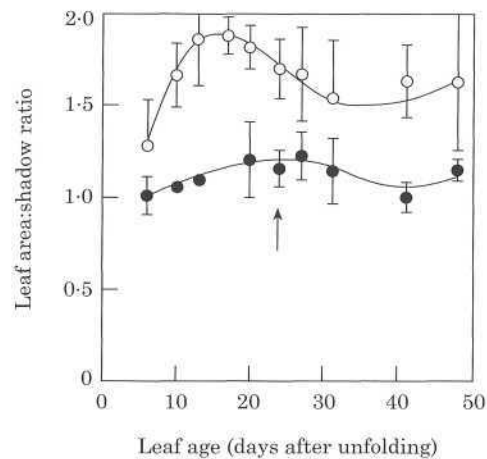


FIG. 4. Changes to leaf cupping during development in *G. hirsutum* (●) and *G. barbadense* (○) leaves. The vertical arrow indicates the day maximum leaf area was achieved. Mean \pm s.d., $n = 4$ for each datum point.

G. barbadense leaves are larger and thinner than those of *G. hirsutum*, with much of the difference in thickness due to a thicker palisade layer in *G. hirsutum* (Figs 1–3). *G. barbadense* also has a lower light-saturated photosynthetic rate (Fig. 8; Sassenrath-Cole *et al.*, 1993; Lu

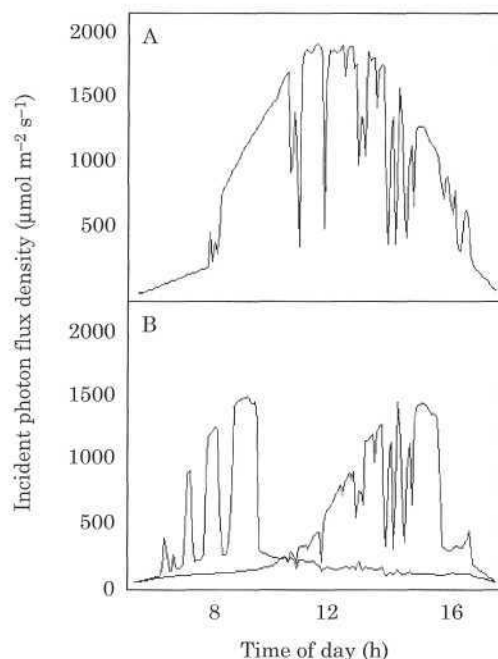


FIG. 5. Photon flux density measured on the adaxial surface of *G. hirsutum* (A) and *G. barbadense* (B) leaves. Sensors were placed either on the midrib (for *G. hirsutum*, A) or on the east- (left trace in figure) and west-facing (right trace in figure) margins of cupped *G. barbadense* leaves (B). Irradiance was measured every 5 s over the course of a 20 h period and 5 min averages were stored in a data logger.

et al., 1997). These observations are precisely what would be predicted from the model of Pachevsky and colleagues (Pachevsky *et al.*, 1995). These authors postulated that a thicker palisade layer would increase the cell area index (the ratio of mesophyll cell surface area to leaf surface area) and allow for an increase in maximum photosynthetic capacity (Pachevsky and Acock, 1998). Therefore, within the parameters of the Pachevsky model, the anatomy of the *G. hirsutum* leaf would appear to be better adapted for maximal photosynthesis than *G. barbadense*.

The differences in photosynthesis represent only a 15 % decrease in maximal photosynthetic activity at saturating illumination between the two species (Fig. 8). However, leaves rarely experience saturating illumination in the mid-South due to haze caused by high humidities, while the period of clear skies and full sunlight is much longer in

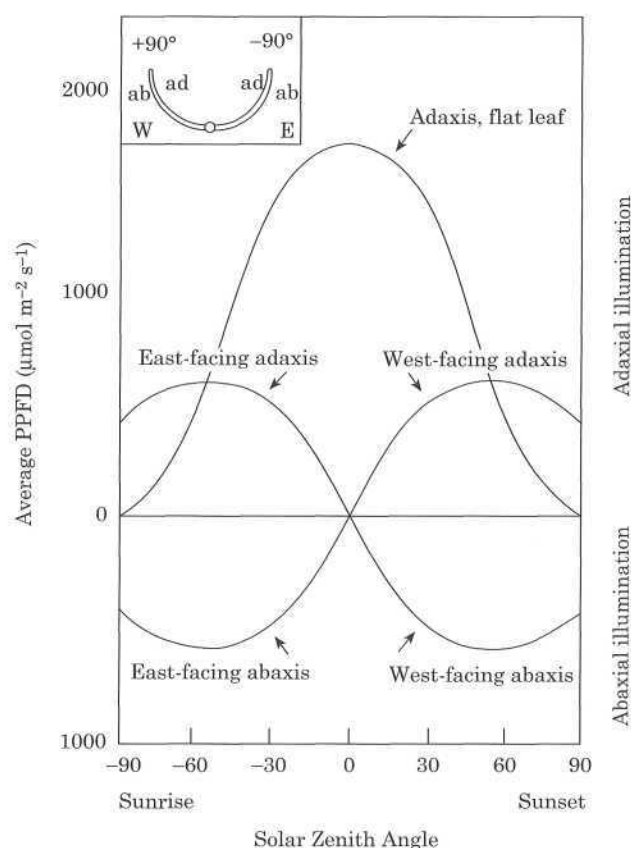


FIG. 6. Predicted diurnal changes in photon flux to cotton leaf surfaces as a function of vertical orientation of leaf surface. The trace labelled 'Adaxis, flat leaf' represents the condition in *G. hirsutum* leaves. The other two traces pertain to the light which would be absorbed by the cupped margins of *G. barbadense*. Inset: model diagram of a *G. barbadense* leaf in cross-section showing the orientation of the cupped leaf margins to the sun. Leaf midrib runs north/south.

Arizona. Therefore, most of the time cotton plants growing in Mississippi are receiving less than $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$, at which PPFD there were no observed differences between the cultivars (Fig. 8). The limitation in available sunlight may be one of the factors restricting the success of *G. barbadense* in the mid-South USA.

If the *G. barbadense* leaf anatomy is not optimized for maximal photosynthesis, then perhaps the forces driving anatomical design have been canopy structure and water

TABLE 1. Stomatal densities, stomatal length, width, and area, and percent of leaf surface occupied by stomata in *G. hirsutum* and *G. barbadense*

Anatomical character	<i>G. hirsutum</i>	<i>G. barbadense</i>	<i>P</i>
Abaxial stomatal density (per mm^2 of leaf)*	219.7 ± 53.0 (15)	391.2 ± 86.4 (15)	<0.001
Adaxial stomatal density (per mm^2 of leaf)*	80.0 ± 25.5 (15)	136.2 ± 37.6 (15)	<0.001
Average stomatal length (μm)	20.3 ± 4.0 (60)	12.3 ± 2.8 (60)	<0.001
Average stomatal width (μm)	4.8 ± 1.3 (60)	2.8 ± 0.9 (60)	<0.001
Average stomatal area (μm^2)	76.4 ± 26.8 (60)	27.4 ± 12.4 (60)	<0.001
Percent of abaxial leaf surface occupied by stomata	1.68	1.07	
Percent of adaxial leaf surface occupied by stomata	0.61	0.37	

Mean \pm s.d., sample size given in parentheses. Statistical differences between means (*P*) were determined using a Student's *t*-test.

* Average of the five measurements taken on each of 40, 46 and 55 d after unfolding (see Fig. 7).

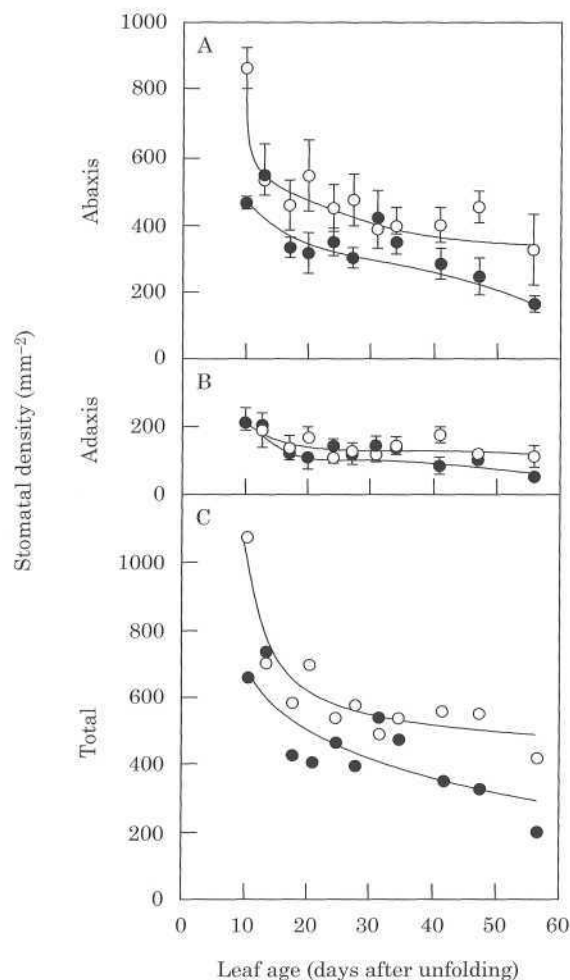


FIG. 7. Stomatal densities on developing leaves of *G. hirsutum* (●) and *G. barbadense* from 10 DAU to 57 DAU (○). A, Abaxial surface; B, adaxial surface; C, total. Mean \pm s.d., $n = 5$ for each datum point.

conservation. Pima leaves have significant three-dimensional cupping (Fig. 3, Sassenrath-Cole, 1995) which decreases the total insolation to the individual leaf surface over the course of a day (Fig. 6), but results in a more even distribution of PPFD among the canopy layers and a greater penetration of sunlight to lower canopy leaves (Sassenrath-Cole, 1995). Lower leaves in a *G. hirsutum* canopy receive less than 1% of the incident PPFD in a mature, closed canopy (Sassenrath-Cole *et al.*, 1993). *G. barbadense*'s leaf curling would also allow the exposed abaxial layers to achieve maximal photosynthesis in the morning and evening when the vapour pressure deficit (VPD) is lowest, while the mid-rib adaxis would perform maximal photosynthesis at noon when VPD is highest. The effect of the high mid-day VPD would be partially offset by the increase in adaxis boundary layer resistance afforded by the leaf cupping. Therefore, it is quite possible that the total canopy photosynthesis of *G. barbadense* over the course of a day is higher than that of *G. hirsutum*, while the total canopy transpiration is lower (transpiration rates of *G. barbadense* are lower than those of *G. hirsutum* on a

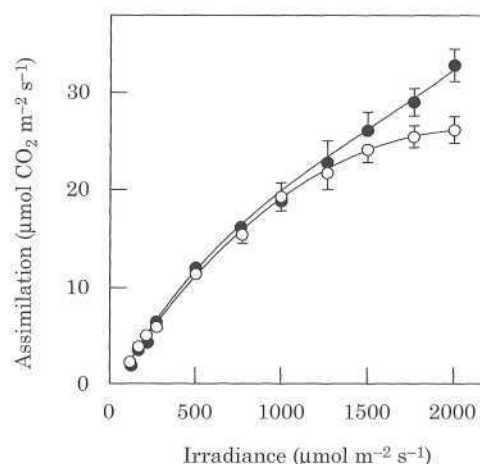


FIG. 8. Light saturation curves of photosynthesis for *G. hirsutum* (●) and *G. barbadense* (○) leaves. Mean \pm s.d., $n = 5$ for each datum point.

leaf area basis—see Lu *et al.*, 1997). Unfortunately, such measurements do not yet exist in the literature.

Another potential consequence of leaf cupping in *G. barbadense* could be protection from photoinhibition. Photosynthesis in *G. hirsutum*, particularly in younger leaves, does not saturate at full sunlight (Fig. 8, Sassenrath-Cole *et al.*, 1996). Photosynthesis in *G. barbadense*, on the other hand, does saturate, therefore it may be necessary for *G. barbadense* leaves to cup to avoid the damaging effects of full sunlight, i.e. photoinhibition. Leaf cupping results in only a portion of the leaf being in direct sunlight at any given period of the day (Fig. 5), and hence would limit the time of exposure to full sunlight, and the potential damage.

Several studies have demonstrated an effect of incident PPFD on leaf development in cotton (Smith and Longstreth, 1994) and other species (Schoch *et al.*, 1975; Lichtenthaler *et al.*, 1981; Buisson and Lee, 1993). In these studies, high-light-grown plants had thicker leaves and a higher stomatal density than leaves on low-light-grown plants. Given that the 'horizontal' and 'vertical' portions of the *G. barbadense* leaves received different levels of light throughout development (Fig. 5), the anatomy and stomatal densities of the two leaf regions were compared. There were no measurable differences in leaf thickness, percent of leaf thickness as palisade layer, or stomatal density between the horizontal and vertical areas of the *G. barbadense* leaves (data not shown). Therefore, even though leaf curling in *G. barbadense* had the potential to cause distinctly different light environments during development for the two leaf regions (Fig. 5), the differences in light quality and quantity had no measurable effect on *G. barbadense* leaf anatomical development.

On an equal leaf area basis, stomatal conductance in field-grown *G. hirsutum* plants is 25–35% higher than that of *G. barbadense* (Lu *et al.*, 1997). This observation may be explained, at least in part, by the fact that *G. barbadense* only has about 60% of the total percent of leaf occupied by stomata on both the abaxial and adaxial surfaces as compared to *G. hirsutum* (Table 1).

Photosynthetic measurements reported in Lu *et al.* (1997) indicate that *G. barbadense* may be particularly sensitive to

high irradiation and high temperatures, as evidenced by a significant midday reduction in photosynthesis. In addition, growth and yield in *G. barbadense* are more sensitive than *G. hirsutum* to water deprivation (Saranga *et al.*, 1998). Breeding efforts have found that increases in yield have occurred concomitant with increases in stomatal conductance and an overall more favourable energy balance (Radin *et al.*, 1994; Lu *et al.*, 1997). Yet in spite of this, transpiration is lower in *G. barbadense* than in *G. hirsutum*, and its leaves (Lu *et al.*, 1997) and canopies (Sassenrath-Cole *et al.*, 1993) are 1 to 1.5°C warmer than those of *G. hirsutum*. The cupping of *G. barbadense* leaves (Fig. 4) may contribute to the dissipation of heat load by keeping only a small proportion of the total leaf surface at full sunlight at any given time (Fig. 5).

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